Reference: *Biol. Bull.*, **151**: 236–246. (August, 1976)

CIRCADIAN RHYTHMS IN CORALS, PARTICULARLY FUNGIIDAE

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During the day, most of the many species of corals which comprise a tropical reef are contracted, and the tentacles are withdrawn within the horney or calcareous skeleton (Abe, 1939; Yonge, 1940). Feeding takes place at night when the tentacles are expanded. However, there are soft and hard corals in which the tentacles are expanded during the day, and in a few species, the tentacles are always expanded unless they are touched or during reproduction (Wells, 1966). To this author's knowledge, the behavior of corals has not been observed previously under either continuous light or constant darkness in the laboratory. Without such observations, it is impossible to determine whether polyp expansion is directly determined by environmental light or whether it is wholly or partly under the control of a circadian rhythm.

The opportunity for making such observations was provided at the shore station which was established at the Banda Islands, Indonesia by the ALPHA HELIX expedition to Indonesia in the spring of 1975. Species of the scleractinian family Fungiidae are particularly common in the Banda region (Wells, 1966). They are large single polyps not attached to the substrate when mature, and hence they can easily be collected without damage. Fungiids were thus particularly singled out for study. The following is a report of the evidence that tentacle expansion and contraction are controlled both by environmental light and darkness and by a circadian rhythm.

Materials and Methods

Coral specimens were collected from the reefs around the Banda Islands of Neira, Gunnan Api and Banda Bessar while snorkeling. The species studied are listed in Table I. Specimens were placed in a pail under water and then brought to the laboratory without exposure to air. Fungiids were picked up from the bottom, to which they are not attached when mature. Specimens of *Tubipora* and *Euphyllia* were dislodged from a colony with a section of pipe or an abalone iron. On returning to the shore station, specimens were immediately transferred to the experimental tank without exposing them to the air. All specimens were assigned a number on collection. All fungiid specimens used in this study have been deposited in the National Museum, Washington, D. C.

Two experimental tanks of 77 l capacity were filled with unfiltered sea water from the inner harbor at Banda Neira. The water was aerated continuously by a stream of bubbles from an air pump. The temperature of the tanks was $28 \pm 0.2°$ C. The sea water was replaced every four to five days, or sooner if it became cloudy. Zooplankton was occasionally added to the tanks as food, but not according to a regular schedule. One of the experimental aquaria was exposed to natural light from a north-facing window just behind it. Since Banda lies close to the equator at

TABLE I

Corals observed in aquaria in the laboratory and the presence of circadiam rhythmicity in the expansion and contraction of their tentacles.

Species	Specimen number	Rhythmicity demonstrated in:		
		LD	DD	LL (low 1)
Fungiidae				
Cycloseris laciniosa Boschma	43	+		
Fungia fungites Linnaeus	4	+		
	25		+	
	26	+		
	29	+	+	
	30	+		
	40	+		
Fungia paumotensis Stutchbury	1	+	+	
	2		+	
	15		+	
	28	+		+
	32		+	
	36	+		
	42	+		
Fungia echinata Pallas	37	+	+	
	39	+	+	
Fungia repanda Dana	5	+		
	13	+	+	
	16	+		+
	27	+		
Fungia concinna Verrill	18		+	
Fungia concinna Verrill var.	3	+		
	10		+	
	41	+		
Fungia actiniformis Quoy & Gaimard	11	0		
Polyphyllia talpina Lamarck	7	0		
	8	0		
	35	0		
Halomitra philippinensis Struder	12	+	+?	
Other Corals				
Euphyllia rugosa Dana		0		
Tubipora musica Linnaeus	1	+	?	
	2	+	?	

4.5° S latitude, this tank was brightly illuminated for most of the day. At the time the observations were made, the sun rose at about 0630 and sank at about 1830 local time. The second tank was completely covered with a triple thickness of heavy black plastic sheeting. A corner of this covering was raised for observation of the coral specimens within, which were briefly illuminated by flashlight. Exposure to the flashlight beam did not cause the tentacles to retract if they were expanded.

Coral specimens in both tanks were examined at hourly or two-hourly intervals between 0600 and 2300 local time each day, sometimes also between 2300 and 0600. The time was recorded and the condition of the tentacles was scored as expanded (EX), partially expanded (PC) or contracted (C). It was not practical to measure

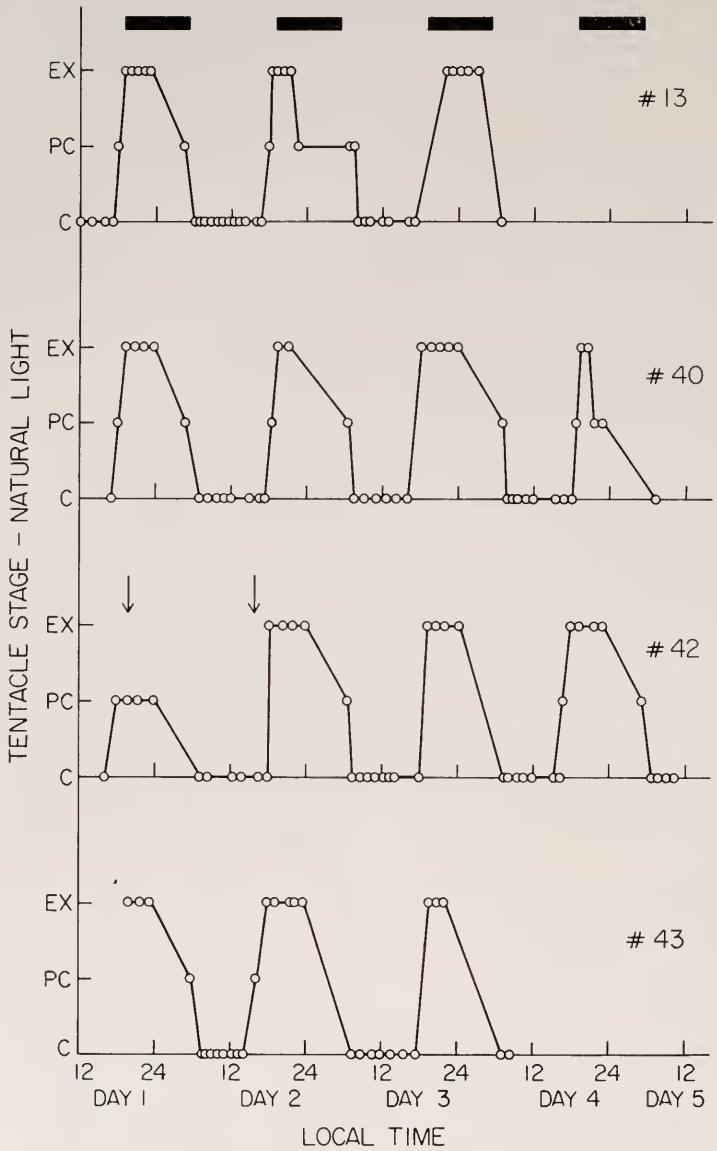

FIGURE 1. The temporal pattern of expansion and contraction of the tentacles in four species of Fungiidae in natural light: No. 13, *Fungia repanda* Dana; No. 40, *Fungia fungites* Linnaeus; No. 42, *Fungia paumotensis* Stutchbury; and No. 43, *Cycloseris laciniosa* Boschma. The tentacle stage was scored as expanded (EX), partially expanded (PC) or contracted (C). Arrows denote the time when zooplankton was added to the aquarium. The dark periods in the environment are shown as black bars on the abscissa. The temperature was kept at 28 ± 0.2° C.

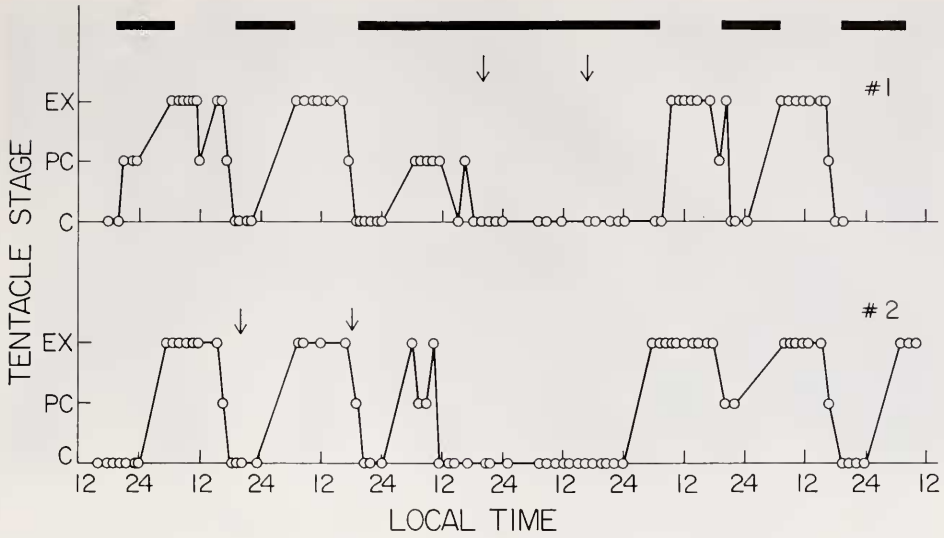

FIGURE 2. The temporal pattern of expansion and contraction of the polyps of two specimens of *Tubipora musica* Linnaeus in natural light, then during 60 hours in darkness, followed by natural light. The tentacle stage was scored as in Figure 1. Arrows denote the time when zooplankton was added to the aquaria. The dark periods are shown as black bars on the abscissa. The temperature was kept at 28 ± 0.2° C.

the length of the tentacles. Usually specimens were first examined for a day or two in the tank exposed to natural illumination and were then transferred during the night to the continuously darkened tank for further observation. A few specimens were observed in continuous light from cool white fluorescent lamps in an incubator at an intensity of 100 lux at 28 ± 0.5° C. Exceptions to this general procedure will be noted. Only specimens in good condition without signs of damage were used. Sand was placed in the tanks for supporting *Tubipora* in an upright position.

In the experiments testing the light sensitivity of *Fungia*, an electronic flash unit (Sun Pac GT3) giving flashes of 1 msec duration, 6000° K color temperature, was fired once. The subsequent behavior of tentacles was observed at intervals of a minute thereafter until the tentacles were completely reexpanded. For photography of tentacles, a Nikon N355 Photomicrographic dark box was attached to the dissecting scope, and the specimens were illuminated with the flash unit described above.

RESULTS

Observations made in the field while snorkeling over the reefs around the Banda Islands during the day confirmed that the majority of both hard and soft corals were in the contracted condition. An enumeration of the species with expanded or contracted tentacles made in the afternoon on two occasions, one at Banda and the other in Weda Bay, both gave an approximate figure of 80% of all species with contracted tentacles. Enumeration at night was attempted from a boat but visibility was insufficient to allow a count. Snorkeling at night in these shark-infested waters is not inviting and was not attempted.

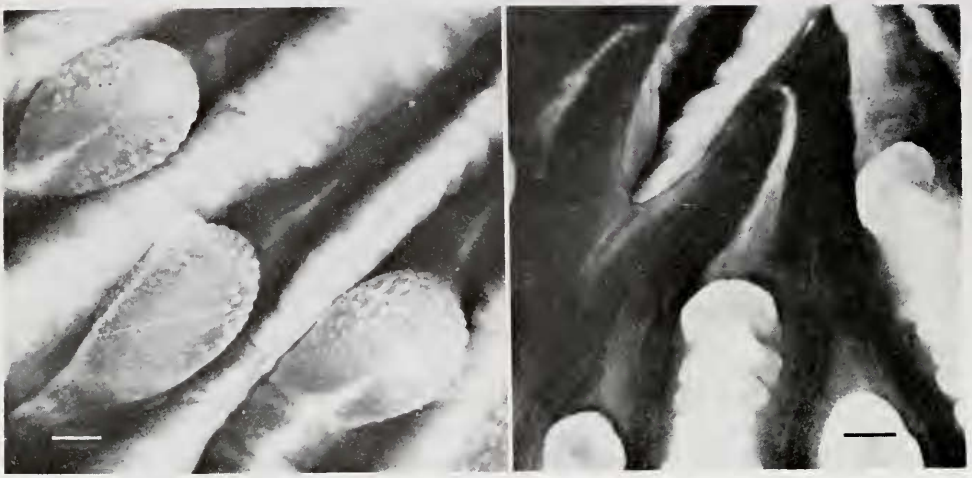

FIGURE 3. Tentacles of *Fungia paumotensis* Stutchbury shown in the expanded (left) and contracted condition (right). The scale bar equals 1 mm.

In the laboratory aquarium under natural illumination, the specimens of Fungiidae behaved in one of two ways (Table I): either the tentacles expanded about one hour after dark and contracted at or somewhat after dawn (Fig. 1), or they remained expanded continuously. Only *Fungia actiniformis* and three specimens of *Polyphyllia talpina* fell into the latter category. One non-fungiid coral, *Euphyllia rugosa*, also remained expanded both day and night under natural light. In none of the specimens of Fungiidae were the tentacles expanded during the day and contracted at night. This behavior was observed in two specimens of *Tubipora musica* (Fig. 2).

Scoring the stage of tentacle expansion was not difficult, since the tentacles of many species of Fungiids are quite long, 3–15 mm, when expanded (Fig. 3). The tentacles of *Fungia actiniformis* are much longer, 50 mm or more. The polyps of *Tubipora musica* showed white against the red skeleton when expanded, while in *Euphyllia rugosa*, the large light-green polyps are easily visible.

The behavior of all the corals in the laboratory under natural light conformed to that observed on the reef. *Fungia actiniformis* was known from previous field studies to remain expanded continuously except during planulation, the only member of the Fungiidae reported to behave in this manner (Wells, 1966). An additional species, *Polyphyllia talpina*, can now be added to this category.

Under the experimental conditions, expanded tentacles of *Fungia* responded to touch by contracting rapidly and were fully reexpanded after 15–20 minutes (Fig. 4). Tentacles did not contract when illuminated for 10–15 minutes with the flashlight used for observations. The tentacles of *Fungia* can respond to light, however. While expanded, three specimens were illuminated by a single flash of high intensity and short duration. All contracted after a minute's latency (Fig. 4) and reexpanded 10–30 minutes later. Contraction of the polyps in bright light can also be inferred from a comparison of tentacle behavior in natural light with that in continuous darkness, as described below.

To determine whether the contraction of the tentacles during the day was the direct result of illumination or might be under the control of a circadian rhythm, specimens were transferred from the aquarium in natural light to the adjacent aquarium darkened with black plastic. The transfer was usually made during the evening to avoid rephasing a rhythm if one were present. Darkening an organism during the light part of a light-dark environmental cycle is known to rephase circadian rhythms in two organisms (Karakashian and Schweiger, 1976), and does so in *Fungia* (Fig. 7). No specimen which had shown alternating contraction and expansion under natural light remained in the expanded state continuously in darkness. Thus light-induced contraction cannot alone be responsible for the contracted state of tentacles during the day. A distinct circadian rhythm was evident in the records of a number of specimens in continuous darkness (Table I and Fig. 5). The period of the rhythm under these conditions appeared to be somewhat shorter than 24 hr, but tentacle behavior proved too erratic to allow accurate determination of period. The tentacles of one specimen of *Fungia paumotensis* (specimen No. 32) behaved arhythmically when first placed in the darkened tank, but later became clearly rhythmic (Fig. 5). Tentacles remained expanded for a considerably longer time in continuous darkness than in natural light (Fig. 5). This observation suggested that light might play a part in determining the temporal pattern of tentacle behavior.

In contrast to the fungiids examined, *Tubipora muscia*, in which the tentacles were expanded only during the day in natural light, showed a single cycle of expansion in continuous darkness, thereafter remaining contracted (Fig. 2). This behavior was not caused by poor condition of the specimens, since they behaved normally on being returned to natural illumination.

Many rhythms have been shown to continue in constant light of low intensity as well as in continuous darkness. Single specimens of *Fungia paumotensis* and *F. repanda* were transferred to constant illumination during a day. In bright con-

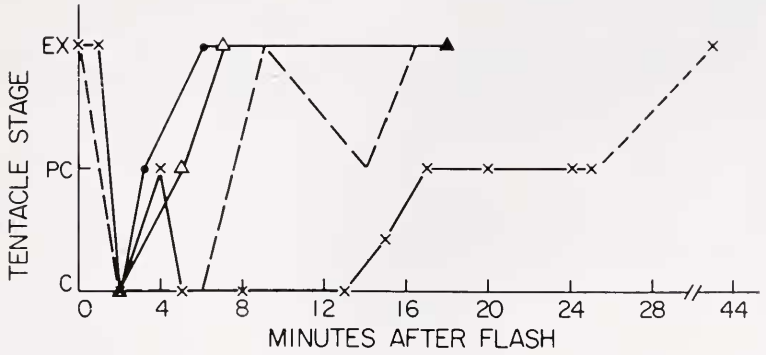

FIGURE 4. The time course of tentacle contraction and expansion following a bright flash, 1 msec in duration, applied at time 0 to specimens with expanded tentacles during the evening in natural light (*Fungia fungites* Linnaeus, open circle; and *F. paumotensis* Stutchbury, open triangle) or in continuous darkness (*F. paumotensis* Stutchbury, x). The dashed line shows the response of *Fungia echinata* Pallas, to touch at time 0. Tentacle stage is scored as in Figure 1.

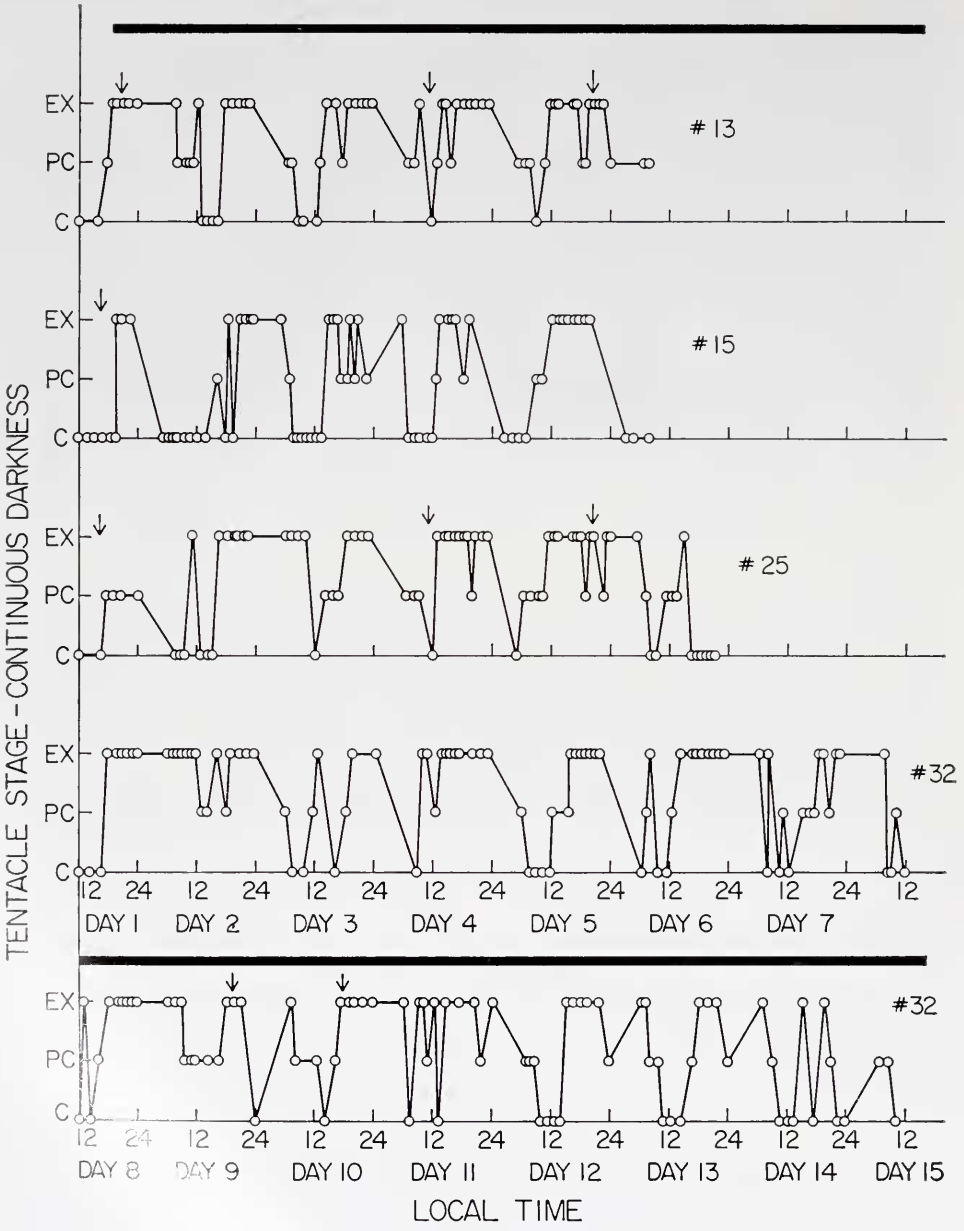

FIGURE 5. The temporal pattern of expansion and contraction of the tentacles in three species of *Fungia* in continuous darkness, as shown by the black bar on the abscissa. No. 13 is *Fungia repanda* Dana; No. 15 and No. 32 are *F. paumotensis* Stutchbury; and No. 25 is *Fungia fungites* Linnaeus. Arrows denote the time when zooplankton was added to the aquarium. Tentacle stage was scored as in Figure 1. The temperature was kept at $28 \pm 0.2°$ C. A circadian rhythm is clearly discernible.

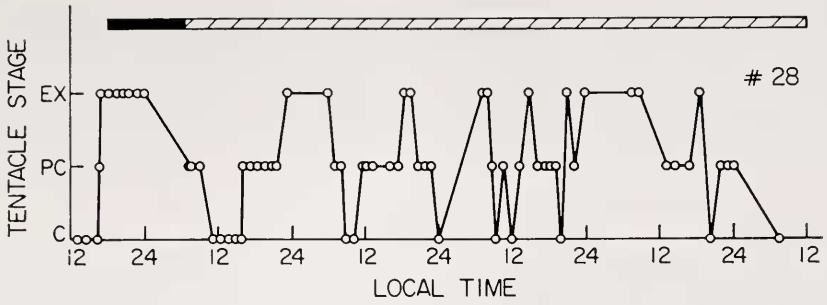

FIGURE 6. The temporal pattern of expansion and contraction of tentacles of *Fungia paumotensis* Stutchbury in natural light for one day, then transferred to continuous light of low intensity (100 lux), as shown by the hatched bar on the abscissa. The temperature was kept at 28 ± 0.5° C. A circadian rhythm is apparent during the first two days in continuous light. Tentacle stage was scored as in Figure 1.

tinuous light, the tentacles remained contracted. However, in light of low intensity (100 lux at 28° C), these specimens displayed a clear circadian rhythm (Fig. 6).

DISCUSSION

The strongly nocturnal behavior of most corals in nature suggests that tentacle expansion might be under the control of a circadian clock, in view of the large number of other nocturnal animals in which such a mechanism has been shown to operate (De Coursey, 1960; Halberg, 1959; Harker, 1954). Among the Coelenterates, however, circadian rhythms have been demonstrated only in the sea-pen, *Cavernularia obesa* (Mori, 1960) and the sea-anemone, *Metridium senile* (Bantham and Pantin, 1950). In both, the rhythmic patterns of body contraction and expansion are somewhat irregular in constant darkness as compared with circadian rhythms in some other organisms under similar conditions, for example in the dinoflagellate *Gonyaulax polyedra* (Sweeney and Hastings, 1957). As in *Fungia*, some specimens of *Metridium* clearly display a circadian rhythm while others do not. In *Metridium* as in *Fungia*, rhythmicity may be obscured at the beginning of exposure to continuous darkness, only to become apparent later in a series of measurements (compare Fig. 5 of this paper with Fig. 8 of Bantham and Pantin, 1950). Since single-polyp Fungiidae are essentially calcified sea anemones, this similarity in behavior is perhaps understandable. In the sea pen, in the sea-anemone and in *Fungia*, strong illumination causes contraction, and hence serves to synchronize behavior with environmental day and night more closely than does the circadian control. No tidal cycles were observed in the corals studied in Banda; however, these corals are never exposed to the atmosphere even at the lowest spring tides.

In corals, the nocturnal activity is correlated with the frequency of zooplankton in the water column. Many more crustaceans were collected in plankton hauls at night than during the day in the Banda region during these experiments, as is generally the case. It is thought that such zooplankton organisms are the principal food of corals (Yonge, 1930, 1968, 1973).

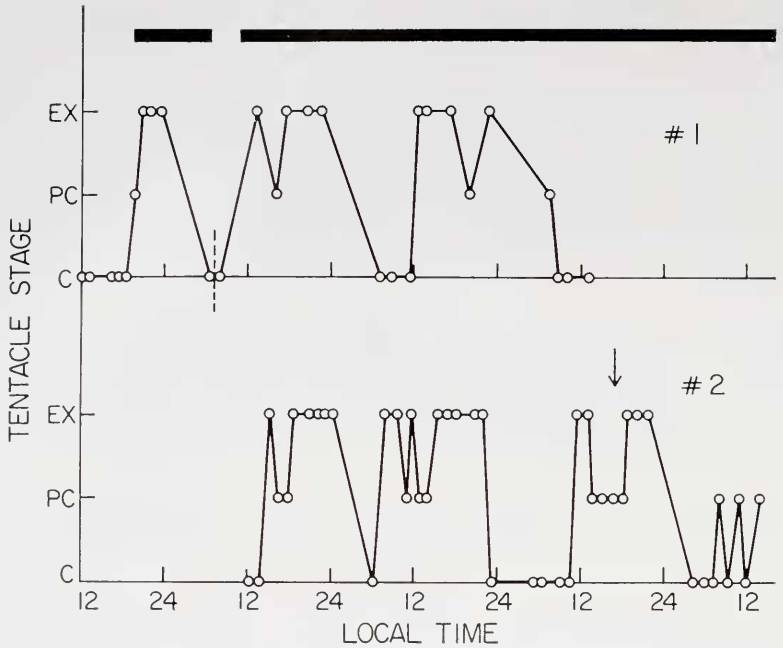

FIGURE 7. The pattern of expansion and contraction of the tentacles of two species of *Fungia* (No. 1, *F. paumotensis* Stutchbury; No. 2, *F. conicinna* Verrill var.) exposed to natural light for one day, then transferred to continuous darkness during the day, at 1030 local time, as shown by the black bar on the abscissa. The arrow denotes the time when zooplankton was added to the aquarium. The tentacle stage was scored as in Figure 1. The temperature was kept at 28 ± 0.2° C. The rhythmic expansion of the tentacles in both specimens after the transfer to constant darkness was phase-shifted, so that the tentacles were now expanded during the local day.

Since fungiids can be maintained in a simple aquarium without running sea water and behave normally for long times, they may become useful experimental animals. They also have the advantage of large size, simplicity of organization and ease of collection. It should prove interesting to analyze their circadian rhythm further. Tentacle expansion is thought to be mediated by contraction of the body muscles which forces water into the tentacles. This contraction must be accomplished by activity in the nerve net. Such activity has been measured directly in *Renilla kollikeri* by insertion of microelectrodes (Anderson and Case, 1975). It is possible that a circadian rhythm in the firing of the nerve net could be detected in *Fungia*. A circadian rhythm in nerve impulses has been documented in the parabolic burster cell of the parieto-visceral ganglion (Strumwasser, 1965) and in the optic nerve (Eskin, 1972) of the sea hare *Aplysia californica*. Such studies might thus be particularly pertinent in analyzing the nature of a circadian clock, in view of the current theories of membrane involvement in this mechanism (Njus, Sulzman and Hastings, 1974; Sweeney, 1974).

The author wishes to express her great appreciation to Professor John H. Wells for identifying all the fungiids used in this study. This study of the ALPHA HELIX East Asian Bioluminescence Expedition was supported by the National Science Foundation under grants OFS 74-01830 and OFS 74-02888 to the Scripps Institution of Oceanography and NSF grant BMS 74-23242 to the University of California, Santa Barbara.

Summary

The temporal patterns of expansion and contraction of the tentacles of seven species of *Fungia*, three other species of the family Fungiidae and two other scleractinian corals have been examined in the laboratory. In six species of *Fungia*, tentacle behavior showed a circadian rhythm, both under natural light and in continuous darkness, while in two species a circadian rhythm was also demonstrated in continuous light of low intensity. *Fungia actiniformis, Polyphyllia talpina* and *Euphyllia rugosa* remained expanded under all conditions. The phase of the tentacle rhythm in *Fungia* could be changed by initiating continuous darkness during the day. Tentacles of *Fungia* were shown to contract in strong illumination. Contraction could be induced by a bright flash of 1 msec duration, after a latency of about one minute. The significance of these findings is discussed.

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